sequence-specific interactions with ORC-like initiators. In prokaryotes, yeast, viruses, and now perhaps mammals, the proteins involved in the initiation process, and the cell cycle control of initiation, may be better understood through analysis of replicator structure (*22*, *26*, *38*, *39*).

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44. We thank E. Epner and M. Groudine for advice regarding the globin locus and for globin sequences and probes; S. Strehl and M. Lalande for sharing the sequence of mitochondrial primers; S. O'Gorman for advice and site-specific recombination reagents; J. Hamlin and M. DePamphilis for sharing data before publication and for insightful comments; J. A. Huberman, M. Mechali, S. Menut, R. Gellibolian, and F. E. Indig for comments on the manuscript; and L. Brody, A. Telling, C. Navarro, and S. Wilson for technical assistance. This work was supported by grants from the NIH (CA48405, GM51104) and the G. Harold and Leila Y. Mathers Charitable Foundation. M.I.A. was supported by a postdoctoral fellowship from the Human Frontiers Science Project Organization and by a special fellowship from the Leukemia Society of America.

13 March 1998; accepted 8 July 1998

BRCA1 Required for Transcription-Coupled Repair of Oxidative DNA Damage

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The breast and ovarian cancer susceptibility gene *BRCA1* encodes a zinc finger protein of unknown function. Association of the BRCA1 protein with the DNA repair protein Rad51 and changes in the phosphorylation and cellular localization of the protein after exposure to DNA-damaging agents are consistent with a role for BRCA1 in DNA repair. Here, it is shown that mouse embryonic stem cells deficient in BRCA1 are defective in the ability to carry out transcription-coupled repair of oxidative DNA damage, and are hypersensitive to ionizing radiation and hydrogen peroxide. These results suggest that BRCA1 participates, directly or indirectly, in transcription-coupled repair of oxidative DNA damage.

An elaborate array of DNA repair systems has evolved in the cell to maintain the integrity of the genetic material. The removal of many types of DNA damage occurs by transcription-coupled repair (TCR), a process in which damage is repaired more rapidly in transcriptionally active DNA than in the genome as a whole (*1*–*5*). This rapid repair is attributable to a faster repair of lesions in the transcribed strand (TS) than in the nontran-

scribed strand (NTS) of active human genes and requires an active RNA polymerase II complex (*6–8*). DNA damage induced by ultraviolet (UV) light is repaired by TCR, as is oxidative DNA damage (including thymine glycols) (*9*). Deficiencies in TCR of oxidative DNA damage have been found in cells from patients with defects in the Cockayne syndrome (CS) group A and group B genes, in cells with certain mutations in the xeroderma pigmentosum (XP) group G gene, and in cells lacking the DNA mismatch repair protein MSH2 (*9–11*).

Germline mutations in the *BRCA1* gene are associated with predisposition to breast and ovarian cancer; they account for approximately half of the inherited cases of these diseases (*12, 13*). The loss of the wild-type *BRCA1* allele during neoplastic transformation in these patients indicates that BRCA1 functions as a tumor suppressor. On the basis of BRCA1's association with Rad51, it has been suggested that BRCA1 acts in concert with DNA repair enzymes to maintain the integrity of the genome during periods of rapid growth (*14–16*). BRCA1 also binds to RNA polymerase II and several transcription factors, including TFIIF, TFIIE, and TFIIH (*17*). This association could reflect BRCA1's proposed function as a transcriptional regulatory protein or a role of BRCA1 in TCR.

To evaluate the role of BRCA1 in TCR, we examined mouse embryonic stem (ES) cell lines that contained one wild-type *Brca1*

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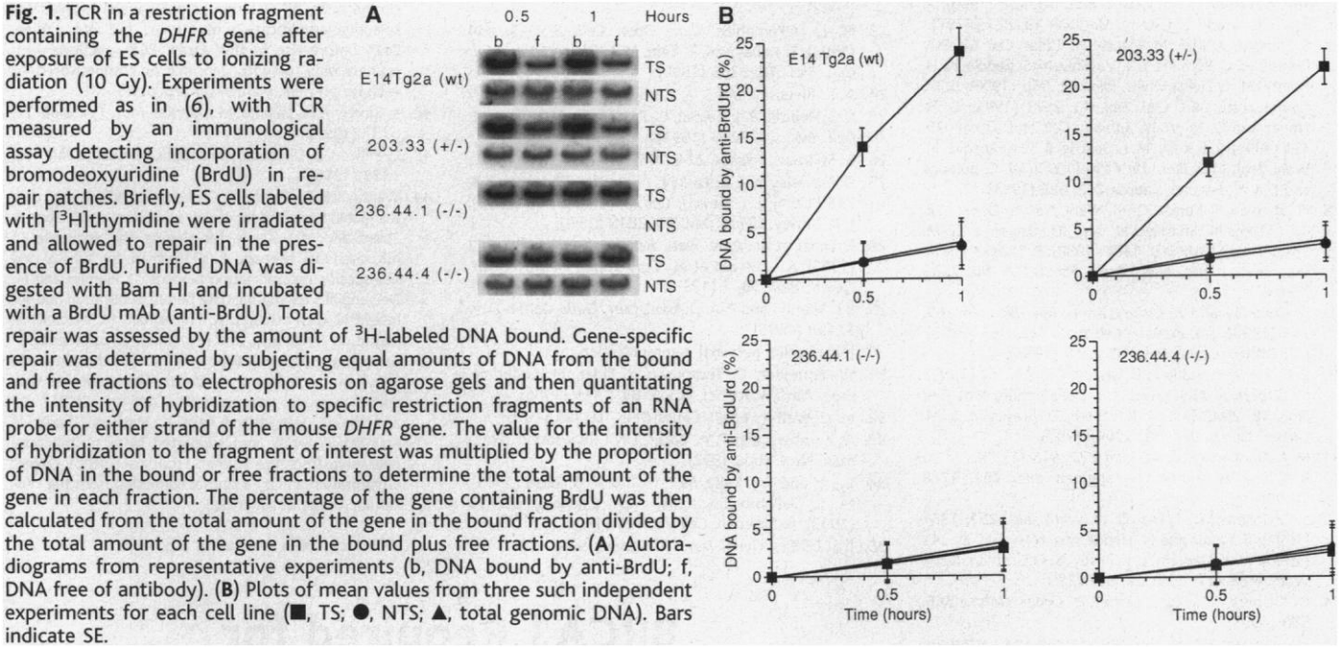

Fig. 1. TCR in a restriction fragment containing the *DHFR* gene after exposure of ES cells to ionizing radiation (10 Gy). Experiments were performed as in (*6*), with TCR measured by an immunological assay detecting incorporation of bromodeoxyuridine (BrdU) in repair patches. Briefly, ES cells labeled with [^{3}H]thymidine were irradiated and allowed to repair in the presence of BrdU. Purified DNA was digested with Bam HI and incubated with a BrdU mAb (anti-BrdU). Total repair was assessed by the amount of ^{3}H-labeled DNA bound. Gene-specific repair was determined by subjecting equal amounts of DNA from the bound and free fractions to electrophoresis on agarose gels and then quantitating the intensity of hybridization to specific restriction fragments of an RNA probe for either strand of the mouse *DHFR* gene. The value for the intensity of hybridization to the fragment of interest was multiplied by the proportion of DNA in the bound or free fractions to determine the total amount of the gene in each fraction. The percentage of the gene containing BrdU was then calculated from the total amount of the gene in the bound fraction divided by the total amount of the gene in the bound plus free fractions. (**A**) Autoradiograms from representative experiments (b, DNA bound by anti-BrdU; f, DNA free of antibody). (**B**) Plots of mean values from three such independent experiments for each cell line (■, TS; ●, NTS; ▲, total genomic DNA). Bars indicate SE.

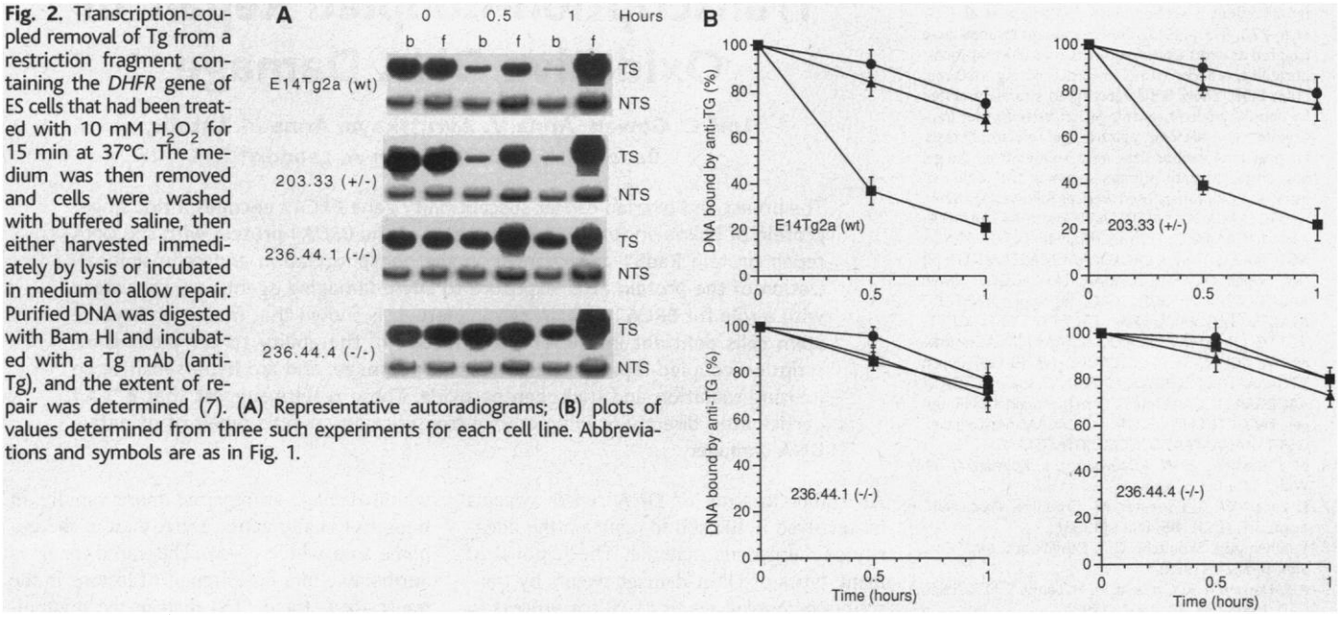

Fig. 2. Transcription-coupled removal of Tg from a restriction fragment containing the *DHFR* gene of ES cells that had been treated with 10 mM H_2O_2 for 15 min at 37°C. The medium was then removed and cells were washed with buffered saline, then either harvested immediately by lysis or incubated in medium to allow repair. Purified DNA was digested with Bam HI and incubated with a Tg mAb (anti-Tg), and the extent of repair was determined (*7*). (**A**) Representative autoradiograms; (**B**) plots of values determined from three such experiments for each cell line. Abbreviations and symbols are as in Fig. 1.

allele and one inactivated allele (*Brca1*$^{(+/-)}$ 203.33), two inactivated *Brca1* alleles (*Brca1*$^{(-/-)}$ 236.44.1 and *Brca1*$^{(-/-)}$ 236.44.4), or two wild-type *Brca1* alleles (E14Tg2a) (*18, 19*). TCR in the actively transcribed dihydrofolate reductase gene (*DHFR*) was measured in cells that had been exposed to 10 Gy of ionizing radiation (Fig. 1). In the parental ES cell line E14Tg2a, the TS was repaired more rapidly than the NTS or the genome overall. The difference between the rates of repair of the TS and NTS was comparable to that observed in normal human fibroblasts. Preferential repair of the TS also occurred in the *Brca1*$^{(+/-)}$ 203.33 cells. In contrast, in *Brca1*$^{(-/-)}$ 236.44.1 and *Brca1*$^{(-/-)}$ 236.44.4 cells, the TS was repaired at the same rate as the NTS and the genome overall.

A defect in the repair of ionizing radiation–induced DNA damage in the BRCA1-deficient cells is consistent with a model in which BRCA1 participates in the repair of DNA double-strand breaks (through its association with Rad51) or repair of oxidized bases, the most abundant class of radiation-induced damage. To determine whether BRCA1 affects the repair of specific oxidative DNA damage, we examined TCR in the cell lines described above using a monoclonal antibody (mAb) that recognizes thymine glycol (Tg), a stable, oxidized base that blocks transcription (*20*). The cells were treated with 10 mM H_2O_2, which produces ~1 Tg per 10 kb of DNA (*21*). The DNA was purified and incubated with Tg mAb, and the antibody-bound DNA was separated from free DNA. Analysis of repair in the *DHFR* gene of the parental E14Tg2a and the *Brca1*$^{(+/-)}$ 203.33 cells by hybridization with strand-specific probes revealed that Tg was removed more rapidly from the TS than from the NTS or the genome overall (Fig. 2). In contrast, there was no preferential removal of Tg from the TS in the two BRCA1-deficient cell lines, although there was no decrease in the overall ability of the cells to remove Tg. These results indicate that (i) preferential removal of Tg from the TS of active genes correlates with TCR of ionizing radiation damage, and (ii) BRCA1 is essential for TCR of oxidative DNA damage, but is not required for global removal of oxidized bases.

We next determined whether the BRCA1-deficient cell lines were defective in repair of UV-induced DNA damage. The ES cells were exposed to UV light (10 J/m^2), and

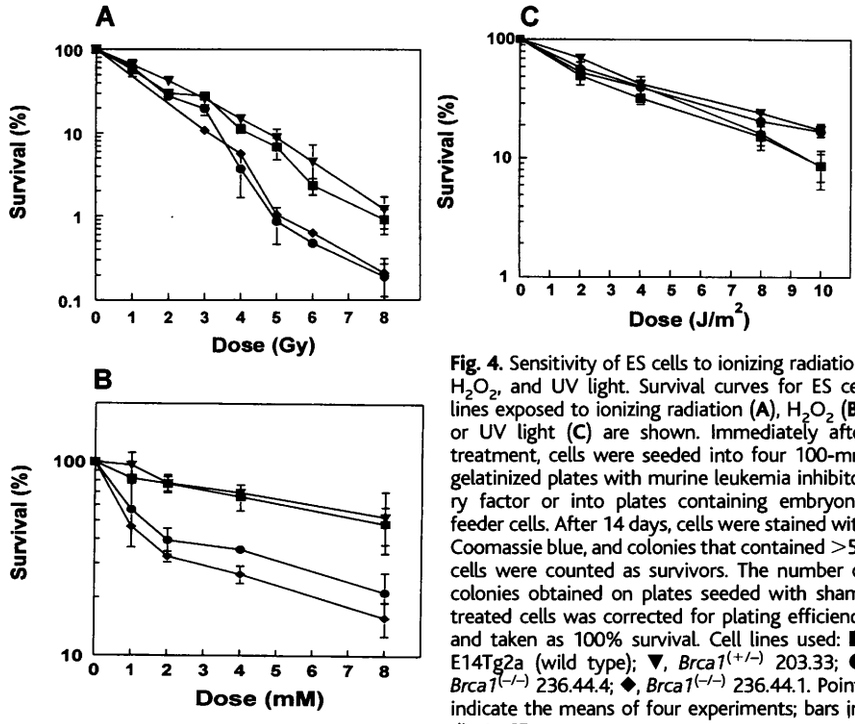

Fig. 4. Sensitivity of ES cells to ionizing radiation, H_2O_2, and UV light. Survival curves for ES cell lines exposed to ionizing radiation (**A**), H_2O_2 (**B**), or UV light (**C**) are shown. Immediately after treatment, cells were seeded into four 100-mm gelatinized plates with murine leukemia inhibitory factor or into plates containing embryonic feeder cells. After 14 days, cells were stained with Coomassie blue, and colonies that contained >50 cells were counted as survivors. The number of colonies obtained on plates seeded with sham-treated cells was corrected for plating efficiency and taken as 100% survival. Cell lines used: ■, E14Tg2a (wild type); ▼, *Brca1*$^{(+/-)}$ 203.33; ●, *Brca1*$^{(-/-)}$ 236.44.4; ◆, *Brca1*$^{(-/-)}$ 236.44.1. Points indicate the means of four experiments; bars indicate SE.

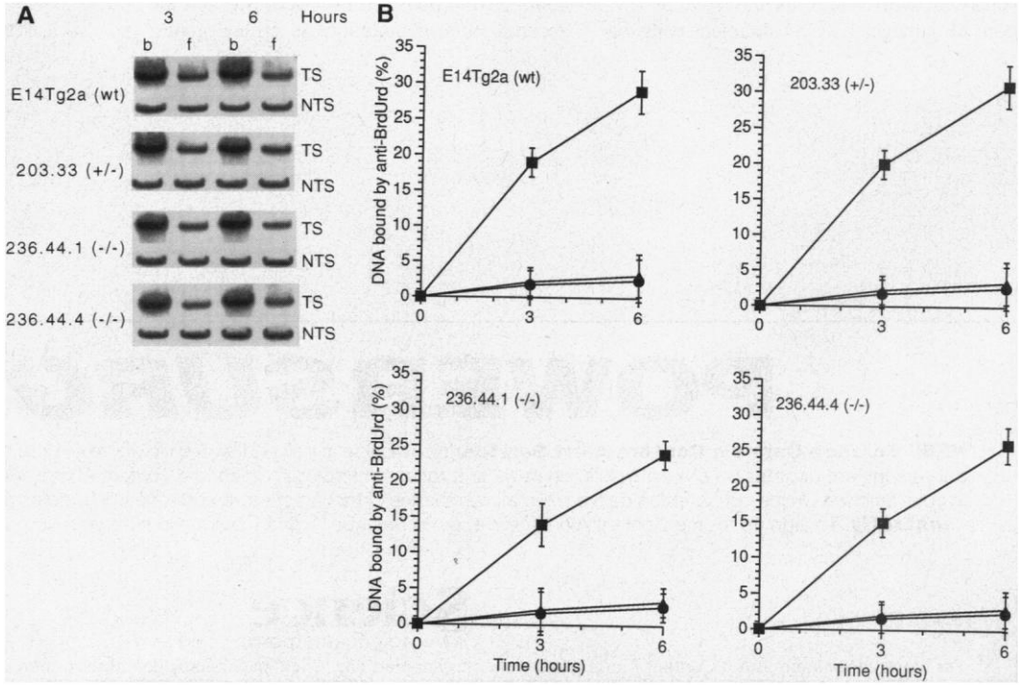

Fig. 3. TCR in a restriction fragment containing the *DHFR* gene after exposure of ES cells to UV light (10 J/m^2). Experiments were performed as in Fig. 1. (**A**) Autoradiograms from representative experiments; (**B**) plots of mean values from three such independent experiments for each cell line, as indicated. Abbreviations and symbols are as in Fig. 1.

repair of the *DHFR* gene was then examined. In the parental E14Tg2a cells, the TS was repaired much faster than the NTS or the genome overall (Fig. 3), and the difference in repair rate was comparable to that observed in normal human fibroblasts. A similar bias toward repair of the TS was seen with the *Brca1*$^{(+/-)}$ 203.33 cells. However, in contrast to the situation with oxidative DNA damage, the BRCA1-deficient cells showed normal preferential repair of UV damage in the TS of the *DHFR* gene. This suggests that although functional BRCA1 is essential for TCR of oxidative DNA damage, it is not necessary for repair of other types of damage in transcriptionally active genes.

Finally, we examined whether the defect in TCR of oxidative damage rendered the BRCA1-deficient cells hypersensitive to agents that produce this damage. The four ES cell lines were exposed to increasing doses of ionizing radiation, and their colony-forming ability was then assessed. When the dose of ionizing radiation was less than 3 Gy, there was little difference in survival between the parental and BRCA1-deficient cells (Fig. 4A). At doses greater than 3 Gy, however, the BRCA1-deficient cells showed reduced survival. At the highest dose examined, 8 Gy, the survival of the BRCA1-deficient lines was reduced by 80% relative to normal cells. Similarly, the BRCA1-deficient lines were more sensitive to H_2O_2 than were the parental lines (Fig. 4B). At the highest dose of 8 mM, there was a 66% reduction in the survival of the BRCA1-deficient lines relative to normal cells. The increased sensitivity of the BRCA1-deficient cells was apparent even after exposure to low concentrations of H_2O_2. Consistent with results on the TCR of UV-induced damage, BRCA1-deficient cells exposed to UV light showed no reduction in colony-forming ability in comparison to parental cells (Fig. 4C).

Our studies show that detection or removal of Tg is severely compromised in BRCA1-deficient cells. However, we cannot distinguish between a direct role for BRCA1 in TCR and a role as a transcription factor essential for the expression of genes whose products are required for TCR of oxidative damage. The TCR removal of Tg, which occurs by base excision repair, also requires the *CSA* and *CSB* gene products, the *XPG* gene product, and the human DNA mismatch repair protein MSH2 (*9–11*). However, unlike BRCA1, these proteins are also required for the TCR of UV-induced damage, indicating a more specific role for BRCA1 in the TCR of oxidative damage.

The hypersensitivity of the BRCA1-deficient ES cells to ionizing radiation and H_2O_2 cannot be explained exclusively by a defect in the repair of DNA double-strand breaks because these lesions are not produced in abundance by H_2O_2 (*22*). In contrast, the increased sensitivity of the *Brca1*$^{(-/-)}$ cells to DNA-damaging agents does correlate with the defective TCR measured in these cells after exposure to each agent. Therefore, it seems likely that defects in TCR alone, or together with some BRCA1-dependent repair pathway as yet undefined, leads to the decreased survival of the mutant cells.

The early embryonic death of *Brca1* homozygote embryos, and the importance of this gene in tumorigenesis, is consistent with a role for BRCA1 and TCR in the growth and development of normal cells (*18, 23*). Deficits in TCR of endogenous oxidative damage could lead to inefficient transcription and the accumulation of mutations in critical genes, leading to inadequate growth during early development or to uncontrolled growth during tumorigenesis.

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24. We thank E. Hicks for assistance with the ES cell lines and I. Mellon for the *DHFR* probes. Supported by NIH grants CA70490 and IP50CA58223 to B.H.K. and CA40453 to S.A.L.

14 April 1998; accepted 1 July 1998